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Oncolytic viruses in cancer immunotherapy

Onkolytické viry v imunoterapii nádorových onemocnění

Bachelor's thesis

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Declaration: I hereby declare that I have compiled this thesis independently, using the listed literature and resources only. The content of this thesis has not been used to gain any other academic title.

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Podpis

Abstract

Oncolytic virotherapy is a field dedicated to exploiting viruses in the battle against cancer, where their specific cytolytic effects are sorely needed. This work focuses on the mechanisms and limitations of oncolytic virotherapy, on the recent advances in the field and on the potential oncolytic viruses hold for the future.

Keywords: oncolytic virus, oncolytic virotherapy, cancer immunotherapy, oncology, tumor

Abstrakt

Onkolytická viroterapie se zaměřuje na využití virů v léčbě nádorových onemocnění, Využívá potenciálu některých virů množit se preferenčně v nádorových buňkách a lyzovat je. Tato práce se zaměřuje na mechanismy a limitace onkolytické viroterapie, na nedávné pokroky v oboru a na budoucí směry výzkumu.

Klíčová slova: onkolytické viry, onkolytická viroterapie, imunoterapie nádorů, onkologie, nádory

List of abbreviations

ADC	- antibody drug conjugate
ATP	- adenosin trisphosphate
BiTE	- bispecific T cell engager
CAR	- chimeric antigen receptor
CPA	- cyclophosphamide
CTLA-4	- cytotoxic T lymphocyte associated protein-4
DAF	- decay accelerating factor
DAMP	- damage-associated molecular patterns
DART	- dual-affinity re-targeting
DC	- dendritic cell
ECM	- extracellular matrix
ER	- endoplasmatic reticulum
GM-CSF	- granulocyte-macrophage colony stimulating factor
HSV	- herpes simplex virus
ICAM-1	- intercellular adhesion molecule-1
IFN	- interferon
IL	- interleukin
MDSCs	- myeloid-derived suppressor cells
NDV	- Newcastle disease virus
NK	- natural killer
NSCLC	- non-small cell lung cancer
OS	- overall survival
PAMP	- pathogen-associated molecular patterns
PD1	- programmed cell death protein-1
PD-L1	- programmed cell death ligand-1
PEG	- polyethylene glycol
Pexa-Vec	- pexastimogene devacirepvec
PKR	- protein kinase R
TAA	- tumor associated antigen
TAP	- transporter associated with antigen processing
TCR	- T cell receptor
TIL	- tumor-infiltrating lymphocytes
TK	- thymidine kinase
TNF	- tumor necrosis factor
TRAIL	- TNF-related apoptosis-inducing ligand
T_{reg}	- regulatory T cell
T-VEC	- talimogene laherparepvec
VSV	- vesicular stomatitis virus

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Introduction

For the past at least fifty years, surgery, chemotherapy and radiation therapy were the staple standard methods of cancer treatment. Even though the immune system of the human body is capable of recognizing and destroying cancer cells, the induction of a specific anti-tumor immune response in clinical setting has always eluded the researcher's efforts - right until the very recent years, when the tide has turned in favour of cancer immunotherapy. That happened mainly thanks to the uncovering of immunosuppressive pathways which tumor cells exploit to escape the immune response. The recent advances and successes of cancer immunotherapy are briefly reviewed in the first chapter of this work.

The majority of this paper focuses on a branch of cancer immunotherapy that is showing an increasing potential. That is the oncolytic virotherapy which harnesses cytolytic machinery of some viruses and their ability to selectively infect and proliferate within tumor cells. The following text describes mechanisms of action of oncolytic virotherapy, its hurdles and limitations. The recent advances including review of clinical trials and the promises of combinational therapy are presented.

1 Advances in cancer immunotherapy

The idea of mobilizing the host's immune system against cancer dates back decades, but in the past such efforts yielded only a limited success. The disappointing results of the studies investigating this promising idea were caused mainly by an incomplete understanding of interactions between the tumor's and the host's immune system, most prominently the tumor's capabilities of immune evasion.

Thanks to the advances in cancer research, it is now evident that various components of the immune system play a crucial role in the recognition and destruction of cancer cells. However, despite the fact that the immune system is capable of elimination of malignant cells, cancer cells are able to evade the immune response by many mechanisms (Sharma et al. 2011). The recognition of multiple tumoral resistance mechanisms in the 1990s (Walunas et al. 1994), and, most importantly, the discovery of immune checkpoint pathways utilized to inhibit the activity of effector immune cells, led to a development of drugs aimed at these immune checkpoints. Ipilimumab, an antibody targeted against CTLA-4 immune checkpoint and antibodies targeting PD1-PD-L1 axis gained approval for clinical treatment of melanoma in 2011 and 2014, respectively (Sharma and Allison 2015). The field of active immunization against cancer is far less successful and many so-called cancer vaccines failed in the phase III clinical trial. The only product that reached the approval based on phase III clinical trial is sipuleucel T, an autologous cell-based therapy approved for the treatment of metastatic prostate cancer in 2010 (Gardner, Elzey, and Hahn 2012).

The success of check-point inhibitors revitalized the field of cancer immunotherapy, which has been experiencing a renaissance in the past decade. Researchers are now investigating the uses of various immune components in the battle against cancer, including naked and conjugated monoclonal antibodies, bispecific antibodies, immune checkpoint inhibitors, pro-inflammatory cytokines, anti-cancer vaccines, adoptive T cell therapies, oncolytic viruses and more. The goal of this chapter is to briefly introduce the most prominent of the emerging cancer immunotherapies, as these will certainly become a part of combinational oncolytic virotherapy treatments.

1.1 Monoclonal antibodies

A range of monoclonal antibodies is developed to target tumor associated antigens or antigens expressed within the tumor milieu such as neovasculature. Native antibodies are able to induce target cell killing mainly by complement-dependent cytotoxicity of antibody - dependent cytotoxicity mechanisms or induce apoptosis of target cell by blocking a specific receptor pathway (Scott, Allison, and Wolchok 2012).

Bispecific antibodies bind to antigens on both tumor cell and effector immune cell (mainly T cell) surfaces, bringing them close together and thus inducing an immune response. Bispecific T cell engagers (BiTE) and dual-affinity re-targeting (DART) are the two most promising classes of bispecific antibodies, with some BiTEs already approved for clinical use (Fan et al. 2015).

Antibody drug conjugates (ADCs) are monoclonal antibodies linked with cytotoxic chemotherapeutics. This approach aims to take advantage of the high antibody-antigen binding specificity, which guarantees specific delivery of cytotoxic agents to tumor cells (Marin-Acevedo et al. 2018). The selection of the target antigen is therefore one of the most important aspects of the ADC therapy. An ideal antigen would be overexpressed by tumor cells, while normal cells would exhibit low or no expression of this particular antigen.

Immune checkpoint inhibitors aim to disrupt the inhibitory effects malignant cells have on immune effector cells by targeting these immune checkpoint pathways, directly binding to the receptors or ligands, effectively blocking the inhibition cascade. Some of these have already been mentioned. Ipilimumab is a CTLA-4 targeted antibody approved for clinical treatment of melanoma, nivolumab and pembrolizumab are PD1-PD-L1 axis targeted antibodies that are approved for clinical treatment of melanoma and non-small cell lung cancer (NSCLC) (Sharma and Allison 2015). Both CTLA-4 and PD-1 immune checkpoint inhibitors are however expected to prove effective against a broader range of malignancies. The major issue of immune checkpoint inhibitor therapy (especially CTLA-4 blockade) are adverse effects stemming from autoimmune reactions (Hodi et al. 2010). The representative examples of approved drugs of each cathegory of monoclonal antibodies are shown in the Table 1.

Table 1: Examples of approved antibody-based oncologic drugs. *Abs* - antibodies, *BiTE* - bispecific T cell engager, *DART* - dual affinity re-targeting, *ADC* - antibody-drug conjugate, *NSCLC* - non-small cell lung cancer.

Drug class	Generic name	Commercial name	Indication	Reference
Native Abs	Rituximab	MabThera®	B-cell malignancies	(Grillo-Lopez et al. 2000)
	Cetuximab	Erbitux®	Colorectal cancer	(S.-F. Wong 2005)
BiTEs	Blinatumomab	Blincyto®	B-cell malignancies	(Przepiorka et al. 2015)
ADCs	Trastuzumab emtansine	Kadcyla®	Breast cancer	(Amiri-Kordestani et al. 2014)
Checkpoint inhibitors	Ipilimumab	Yervoy®	Metastatic melanoma	(Lipson and Drake 2011)
	Nivolumab	Opdivo®	Metastatic melanoma, NSCLC	(Raedler 2015; Kazandjian et al. 2016)
	Pembrolizumab	Keytruda®	Mismatch repair-deficient solid tumors	(Prasad, Kaestner, and Mailankody 2018)

1.2 Cytokine therapy

Pro-inflammatory cytokines and chemokines attracting immune effector cells compose another point of interest for cancer immunotherapy. In particular, IL-12, which has anti-tumor effects thanks to its ability to activate NK and T cells, has been proposed as a potential anti-tumor agent (Lasek, Zagożdżon, and Jakobisiak 2014). IL-2 (Tomala and Kovar 2016) and IL-15 (Stephenson et al. 2012) have been investigated similarly as they act as an immune system enhancer. The major issue of cytokine therapy is a successful delivery of the cytokines to tumor bed, as systemic administration is usually associated with high toxicity. Genetically modified

viral vectors are used for this purpose (Lebel et al. 2016). Note that viral vectors are replication-incompetent, which distinguishes them from conventional oncolytic viruses. Linkage of cytokines to specific monoclonal antibodies is another approach (immunocytokines).

1.3 Adoptive T cell therapy

Adoptive T cell therapy represents, next to immune checkpoint inhibitors, the most promising immunotherapy. It is now evident that lymphocytes are capable of finding and eradicating tumor cells and adoptive T cell therapy aims to put the anti-tumor properties of lymphocytes to fight against cancer cells.

Tumor infiltrating lymphocytes (TILs) are T lymphocytes carrying specific receptors against tumor antigens. In TIL therapy, TILs are first harvested from a surgically removed part of the patient's tumor, then expanded *ex vivo* in laboratory conditions, while being grown with various proinflammatory and immunostimulatory cytokines to reverse the inhibitory properties of some lymphocytes (Gilham et al. 2015). After substantial population of T lymphocytes is grown, TILs are reinfused back into the patient. Despite the very impressive results of TIL therapy (Prickett et al. 2016), a number of challenges need to be resolved before this approach can be implemented as a commonplace cancer treatment. Adoptive TIL therapy is very time consuming and financially demanding. Furthermore, adoptive TIL therapy has so far only been successful against melanoma and no other malignancies. It is speculated that the high mutational burden of melanomas compared to other malignant populations contributes to this fact (Hinrichs and Rosenberg 2014). The advantages of TILs are their relatively low toxicity and their heterogenous specificity, which makes it harder for tumor population to accomplish immunological escape.

Chimeric antigen receptor T cells (CARs) are genetically modified T cells produced by inserting CAR genes to the lymphocyte genome via viral vectors. CAR are essentially monoclonal antibodies functioning as the T cell receptor with a high binding specificity to selected tumor antigens and can therefore bypass the need of antigen-specific MHC recognition. Adoptive CAR therapy yields impressive results for hematological cancers but has yet to show a successful application in solid tumors (Yu et al. 2017). The most severe adverse effect of CAR therapy is

the relatively high toxicity culminating in the so-called cytokine release syndrome (CRS). This is a potentially fatal condition caused by the massive release of cytokines (Lee et al. 2014). Other disadvantages include difficulties of targeting CARs to tumor sites, immune suppressive tumor microenvironment and lack of ideal tumor antigens.

TCR gene modified T cell therapy utilizes genetically modified T cells, which has been inserted with a modified TCR gene which recognizes specific tumor neoantigens presented by the HLA molecules. A potentional advantage of TCR gene therapy over CAR is that modified TCR can be targeted against intracellular neoantigens presented on HLA, whereas CAR can only target surface antigens. Probably the biggest challenge of this approach is the selection of antigens and successful long-term expression of the modified TCR gene (Govers et al. 2010).

1.4 Cancer vaccines

Similarly to prophylactic vaccines against infectious agents, therapeutic cancer vaccines seek to induce an antigen-specific immune response by delivering and presenting tumoral antigens to host's immune cells. Specifically, they are designed to enlarge the T cell populations capable of recognizing and eradicating malignant cells. Cancer vaccines are classified by their method of antigen delivery: tumor cell vaccines (K. Zhang et al. 2017), genetic vaccines (Guo et al. 2013), dendritic cell vaccines (Sabado and Bhardwaj 2013) and peptide vaccines (Ueda et al. 2017), which count among the most prominent.

The major obstacle to the successful utilization of cancer vaccines is the selection of a suitable tumor antigen. Such an antigen should be overexpressed on malignant cells, but normal cells should evince low or no expression. It also has to be noted that tumor cells are often heterogenous and so an immune response against one antigen doesn't guarantee an immune response against the whole tumor. Moreover, tumor cells can mutate in the genes encoding the particular epitopes or stop expressing the antigen altogether, thus evading the immune response (Fenoglio et al. 2013). Finally, in order to be successful, cancer vaccines have to break the immunosuppressive tumor microenvironment, lest the immune response be effectively dampened.

1.5 Oncolytic viruses

This very promising emerging cancer immunotherapy utilizes viruses with natural tropism for malignant cells, which are capable of debulking the tumor beds and inducing an anti-tumor immune response. The rest of this work is focused on this approach.

2 Oncolytic virotherapy

In 2011, Hanahan and Weinberg described the so-called hallmarks of cancer, such as continuous proliferation, disruption of cellular apoptotic apparatus and defective immunological pathways (Hanahan and Weinberg 2011). Many of those characteristics make malignant cells an ideal target for a variety of viruses, which prefer to replicate in such cells, effectively killing them. Thanks to their natural tropism for cancer cells and their lytic activity, these viruses were dubbed oncolytic viruses and researchers had been trying to employ them in the battle against cancer for almost a century, but oftentimes with disappointing results.

However, ever since more knowledge about the mechanisms of oncolytic virotherapy and interactions of cancer cells and host's immune system has been uncovered, the tide has turned in favor of researching new immunotherapies involving oncolytic viruses. In particular, the approval of Talimogene laherparepvec (T-VEC) in 2015 as the first oncolytic agent in clinical use in the US and Europe (Ledford 2015) has boosted the interest in developing new oncolytic viruses.

The goal of this chapter is to review mechanisms and limitations of oncolytic virotherapy, as well as to present recent new approaches of improving oncolytic viruses and finally to show some of the most promising virotherapies in clinical development.

2.1 Mechanisms and limitations of oncolytic virotherapy

The aim of this chapter is to clarify the exact mechanisms of oncolytic activity of oncolytic viruses as well as to present the obstacles and challenges that stand before the modern oncolytic virotherapy.

The most defining characteristic of oncolytic viruses is their ability to infect and kill cancerous cells, but not normal tissue. Their natural tropism for malignant cells can be explained by the overexpression of entry receptors, which viruses use to attach and get into the cytoplasm, by cancer cells and also by the favorable environment for viral replication established by the "hallmarks of cancer" as noted above.

For the most part of the 20th century, oncolytic viruses were attractive because of their ability to directly replicate in cancer cells and killing them afterwards. However, in the late 1990s, it started to be apparent that, sometimes, viral infections induced antigen-specific immune responses against tumors and oncolytic viruses thus effectively acted as cancer vaccines (Toda et al. 1999). Indeed, in the following years, the induction of immune response became a much more appreciated trait of oncolytic virotherapy and, today, its importance eclipses even that of a direct cell killing.

The nature of cellular death plays a role in the magnitude of induction of immune response. For example, necrosis is more immunostimulatory than apoptosis. During stress-induced cellular death, the so-called damage-associated molecular patterns (DAMPs) are released from the dying cell and are subsequently picked by the immune cells, which become activated and induce an inflammatory response. Some examples of DAMPs are ATP, HMGB-1, calreticulin or uric acid. Moreover, such destroyed cells expose neoantigens, which were before inaccessible for the innate immune cells. These neoantigens can then be recognized by the cells of the immune system and lead to a new antigen-specific immune response even in the distant tumor sites which were not infected by the virus. The induction of the so-called immunogenic cell death in tumors which induces a tumor-specific immune response seems to be the major mechanism of antitumoral activity of oncolytic viruses. A simplified scheme of the effects of oncolytic viruses can be seen in Figure 1.

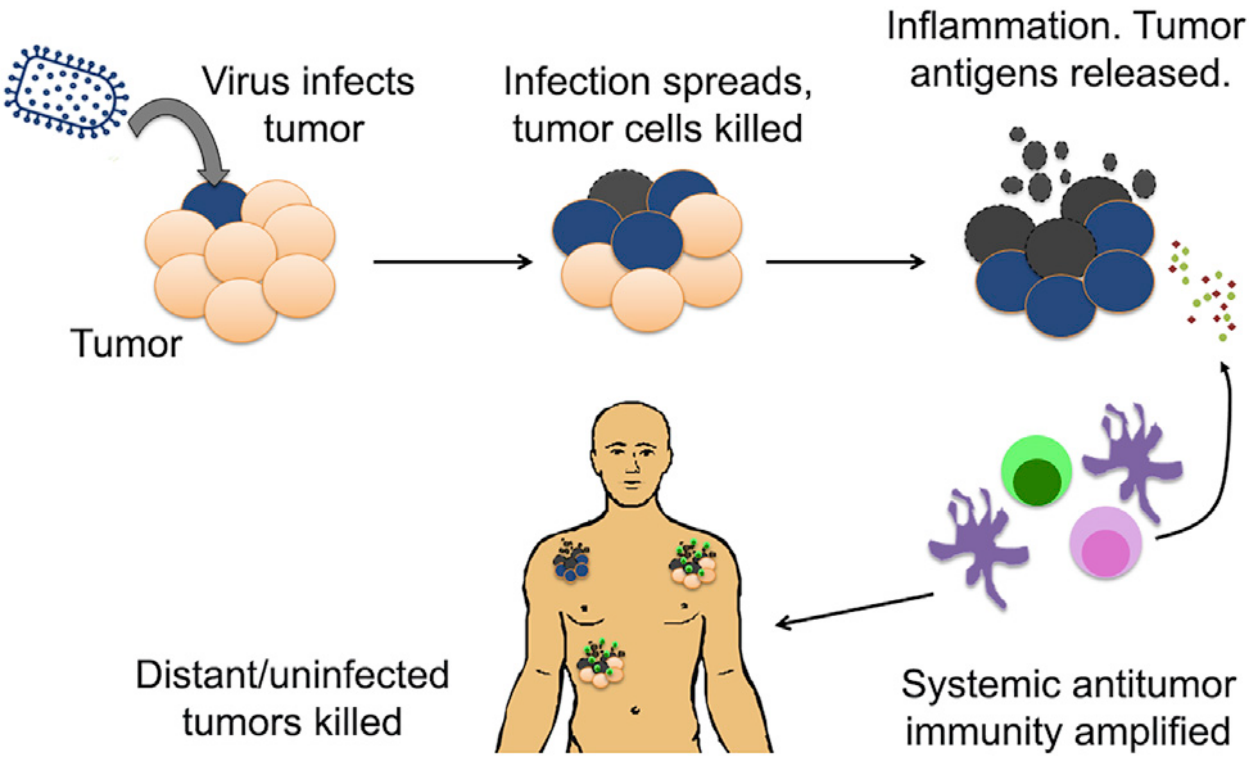

Figure 1: Activities of oncolytic virotherapy. The approach takes advantage of natural tropism of oncolytic viruses against tumor cells and combines both their cytolytic and immunogenic activities. Adopted from (Russell and Peng 2017).

Oncolytic viruses can sometimes infect neighbouring cells that are not malignant but contribute to the immunosuppressive microenvironment in the tumor (e. g. myeloid-derived suppressor cells, MDSCs). These cells can also by killed by the so-called bystander effect, when the released granzymes and perforines from cytotoxic T cells, originally meant for cancerous cells, reach the neighbouring tissue. Increased concentration of immunostimulatory and cytotoxic cytokines also contributes to this effect (Schietinger et al. 2010).

Some oncolytic viruses were also found to target tumor vasculature, infecting endothelial cells and causing thrombosis in tumor vessels (Breitbach et al. 2011; Breitbach et al. 2013).

In addition, oncolytic viruses induce an innate immune response as they carry the pathogen-associated molecular patterns (PAMPs), for example, double-stranded viral RNA. The recognition of PAMPs by their receptors (for example TLR or RLR receptors) in both infected cells and immune cells leads to an inflammatory response, which is in turn important for establishing a stable antigen-specific immune response, because the recognition of PAMPs, DAMPs and proinflammatory cytokines guarantees the activation of costimulatory signals to the

effector immune cells and helps to break the immunosuppressive tumor microenvironment (Maisonneuve et al. 2014).

The most important of the innate immune responses against viral infection is the Type I interferon (IFN) pathway. After the infected cells recognize PAMPs by their PAMP receptors, they produce and release type I IFNs, which activates PKR (protein kinase R) in neighbouring cells. If PKR recognizes dsRNA or other viral PAMPs, it terminates cellular proteosynthesis, effectively killing the cell. However, since PKR pathway recruits p53 and stops cellular proliferation, it is often defective in cancer cells (Clemens 2004).

The activation of anti-viral IFN pathway by the cancer cells therefore acts as a double-edged sword; while its activation leads to viral clearance, it also stops cellular proliferation and induces inflammatory response. The dysregulation in the IFN pathway on the other hand makes it easier for oncolytic viruses to replicate in the tumor bed. The mechanism of facilitated viral infection of cells with defective IFN pathway is depicted in Figure 2.

In short, the oncolytic activity of oncolytic viruses consists of 4 parts: (i) direct cytolytic effect on malignant cells, (ii) the release of proinflammatory and cytotoxic cytokines, (iii) activation of innate immune response, which, among other things, recruits NK cells that preferentially kill cells with downregulated MHC expressions, as is common for cancer cells and, finally, (iv) by inducing antigen-specific adaptive immune response recruiting $CD4^+$ and $CD8^+$ T cells (Cassady et al. 2016).

Thanks to the ongoing attenuation of viral strains and discoveries of new, safer oncolytic viruses, the main struggle of oncolytic virotherapy in the 20^{th} century - uncontrolled virulence with fatal adverse effects - is almost resolved. Nowadays, oncolytic virotherapy is stunted mainly by the premature viral clearance. In other words, it is difficult to find balance between the anti-tumor responses of the host's immune system and its anti-viral activity. The immune system has to be potent enough to fight malignancies, however its activity sets back or downright prevents viral infection.

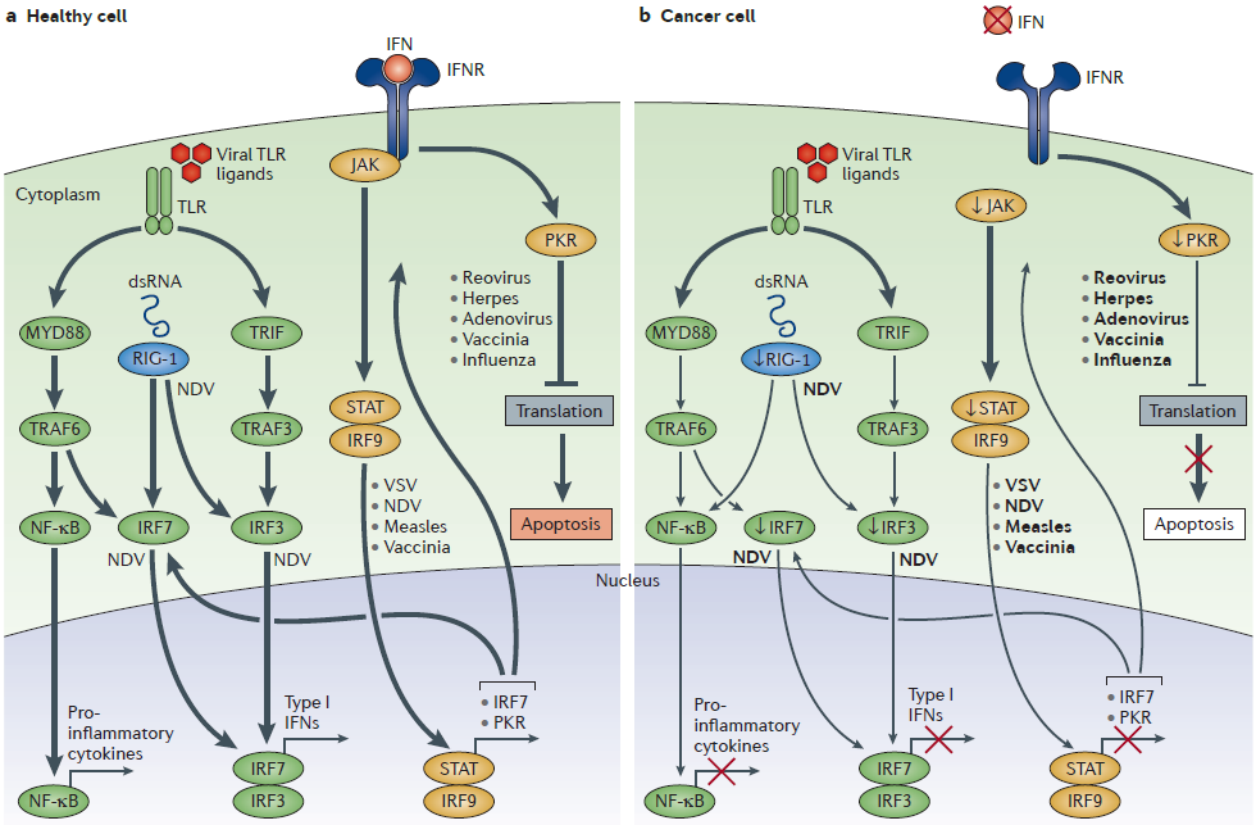

Figure 2: Facilitated viral infection in cells with defective immune pathways. (a) In healthy cells, detection of viral proteins or nucleic acids by Toll-like receptors (TLRs) or RIG-1 like receptors (RLR) activates a signalling cascade that leads to upregulated expression of proinflammatory cytokines and type I interferons. IFNs are autocrine and paracrine mediators that activate Janus kinase (JAK) through the IFN receptor (IFNR). JAK in turn activates STAT cascade inducing the expression of protein kinase R (PKR). PKR, activated by viral nucleic acid, aborts cellular proteosynthesis and promotes apoptosis, thus limiting viral spread. **(b)** In cancer cells, elements of immune pathways are defective or downregulated. In such cells, it is harder to detect viral infection, because TLRs and RLRs are often downregulated. Furthermore, type I IFN expression is often aborted in malignant cells as IFN cascade promotes cellular apoptosis. Oncolytic viruses exploit this characteristic of cancer cells for easier infection and replication. In the depiction, various types of viruses are depicted close to the elements that are known to be responsible for viral elimination. *IFN* – interferon, *MYD88* – myeloid differentiation primary response protein, *NDV* – Newcastle disease virus, *STAT* -signal transducer and activator of transcription, *TRAF* – TNF-associated factor, *VSV* – vesicular stomatitis virus. Adopted from (Kaufman, Kohlhapp, and Zloza 2015).

This problem is the most apparent obstacle of the systemic delivery of oncolytic viruses. Intratumoral injections of viruses are common practice, but, if a systemic, intravenous delivery could be achieved, the virus would potentially be able to infect all disseminated tumor sites, which would greatly enhance its efficacy. Moreover, visceral or bone-residing tumors may not be accessible for intratumoral injection. When administrated intravenously, the dose of the active virus particles is greatly diluted and then further reduced by sequestration in liver and spleen. Innate immune mechanisms also work to get the virus out of bloodstream, eliminating

a number of viral particles by antibodies and complement proteins. Moreover, rapid viral clearance occurs if pre-existing virus-specific antibodies are present in the blood. That is often the case of viruses that cause common infections, which are often contracted at early age (HSV, reovirus) or of viruses that people are getting vaccinated against, such as measles virus.

The size and heterogenicity of the tumor also pose a barrier to a successful virus distribution. Stromal cells, for example tumor associated fibroblasts, can get infected but the virus doesn't undergo the replication cycle in them. Such cells therefore act as decoys, further reducing the dose of viral particles competent to infect tumor cells (Lopez et al. 2009).

A some studies with surprising results made clear that some tumors mutated so that they can upregulate the IFN pathway without suffering from any of its anti-tumor effects (Khodarev, Roizman, and Weichselbaum 2012). Such tumor cells are equipped with a potent anti-viral response, which doesn't reduce their proliferation potential, and that makes oncolytic virotherapy unsuitable for treating such malignancies.

Finally, even if a viral infection successfully induces an antigen-specific adaptive immune response, there is a concern that the immune response would be targeted primarily against the immunodominant viral antigens and this anti-viral response would eclipse any commencing anti-tumor response (Cassady et al. 2016).

2.2 New approaches in oncolytic virotherapy

The goal of this chapter is to briefly review various modern ways of overcoming the obstacles associated with viral therapy and methods for improving oncolytic virus efficacy. In short, these approaches focus on four aspects of virotherapy: (i) attenuation of oncolytic viruses - adapting viruses so that they target preferentially cancer cells while omitting normal tissue, thus increasing their safety, (ii) enhancing the cytolytic activity of oncolytic viruses, (iii) increasing the immunostimulatory potential of virotherapy and finally (iv) improvement of viral biodistribution.

Oncolytic viruses can be attenuated in a number of ways. Most of them focus on improving their ability to replicate in malignant cells, but worsening their ability to infect

normal tissue, thus further specializing the virus strains that already possess natural tropism for cancer cells.

One of such ways is the so-called directed evolution - serial passaging of viruses in human tumor cell lines. The nature of RNA viruses is especially suitable for this approach, while DNA viruses are difficult to adapt this way, as the rate of mutagenesis of the viral DNA genome is much lower than that of RNA viruses (Sanjuán et al. 2010). After a successful parallel passaging of viruses in tumor cell lines, their genomes are sequenced and common mutations increasing virus' specialization to cancer cells are sought after (Sanjuán and Grdzelishvili 2015). In one such study, different adenovirus serotypes were passaged in human colon cancer cell lines, resulting in the emergence of a virus strain with a highly improved replicative potential in cancer cells - ColoAd1 (Kuhn et al. 2008).

Some viruses can be specifically engineered to target surface molecules overexpressed on cancer cells as their entry receptors. For example, Ad5/3-δ24 has been engineered to bind integrins overexpressed on the cells of ovarian carcinoma (You et al. 2001) and measles virus has been engineered to target the carcinoembryonic antigen (CEA) a common surface receptor of various cancers (Hammond et al. 2001).

Another approach is to impair viral ability to block cellular anti-viral responses, which are normally active in healthy cells but dampened in cancer cells. HSV-1 was attenuated this way by the deletion of ICP34.5 and US11 genes, which participate in blocking of PKR phosphorylation (Poppers et al. 2000). Such virus strains can then replicate only in cells with broken PKR pathway. Similarly, B18R gene of vaccinia virus blocks Type I IFN signaling. By the deletion of B18R, vaccinia virus can only competently replicate in cancer cells with defective IFN signaling pathway (Kirn et al. 2007). Adenoviral E1B protein can bind and inactivate p53. Both ONYX-15 and H101, which are adenoviruses used in clinical trials, have deletion in E1B, decreasing their potential to replicate in healthy cells which undergo p53-mediated apoptosis after being infected (Heise et al. 1997; Yuan et al. 2003). On the other hand, nearly a half of all the human cancers have defective p53 pathway, making them susceptible to viral infection.

Viruses can be further specialized by making their replication cycle dependent on tumor environment. For example, the deletion of TK (thymidine kinase) gene in vaccinia virus, renders vaccinia virus to only be able of replication in cells with high concentrations of nucleotides,

which means actively dividing cells (Buller et al. 1985; Guse, Cerullo, and Hemminki 2011). The rate of gene expression can be changed by putting particular viral genes under the control of specific promoters, that are more active in cancer cells. For example, promoters of the prostate-specific antigen (PSA), which is upregulated in prostate cancer cells, or human telomerase transcriptase (TERT), which is an enzyme active in most of human cancers, can be inserted in adenoviruses to control the expression of the E1A gene (Y. Chen et al. 2001; Chang et al. 2009). E1A can also be put under the control of the hypoxia-induced transcription factor HIF-1α, making them preferentially replicating in hypoxic environments, such as the interiors of most tumors (Post et al. 2007).

Finally, viruses can be genetically modified to encode the so-called miRNA targeting sequences (miRTS) in their 3' untranslated regions (UTR). The miRTS bind cellular microRNAs, repressing the viral replication. Measles virus was modified this way to target glioma cells with downregulated miR-7 expression, while blocking replication in normal neurons with upregulated miR-7 expression (Leber et al. 2011).

Methods of improving the cytolytic activity of viruses are also being developed. Gene sequences, that are inserted into viral genomes and, that after expression, make cells more susceptible to cellular death or clearance by the immune system are called suicide genes. A number of those have been successfully inserted in oncolytic viruses with proven efficacy effects.

For example, oncolytic viruses can be engineered to express Fas ligand (Sathaiah et al. 2012), p53 (Van Beusechem et al. 2002) or pro-apoptotic TRAIL or TNF-alpha (Sova et al. 2004; Hirvinen et al. 2015). One of the gene products with direct cytotoxic effects is, for example, the adenovirus death protein (ADP). ADP participates in final stages of adenoviral infection, it has cytolytic effects and is crucial for the release of viral particles. Overexpression of ADP in oncolytic adenoviruses can thus increase their efficacy (Doronin et al. 2000). Another incorporated cytotoxic suicide gene is the bacterial cytosine deaminase (CD), which transforms 5-fluoro-cytosine into cytotoxic 5-fluoro-uracil (Freytag et al. 2003). Oncolytic viruses can also be engineered to target tumor vasculature by expressing VEGF inhibitors (Z. Zhang et al. 2005; Gholami et al. 2014).

As is true for the most of immunotherapies, the ultimate goal of oncolytic virotherapy is to trigger the antigen-specific adaptive anti-tumor immune response of the host's immune system. Today, there are a number of ways of improving the immunostimulatory effect of oncolytic viruses. Most of them consist of inserting genes encoding proinflammatory cytokines or T cell costimulatory molecules into viral genome.

One of the most common inserts is the gene for GM-CSF. GM-CSF attracts DCs, which migrate to tumor bed and load and subsequently present TAAs to effector T cells. GM-CSF had been inserted, for example, into HSV-1 oncolytic viruses, most notably T-VEC. Moreover, T-VEC has a deletion of the viral ICP47 gene (Rehman et al. 2016). Normally, ICP47 blocks TAP, which is a transporter delivering degraded peptides from cytoplasm into the ER, where they bind to MHC glycoproteins. Deletion of ICP46 should thus increase antigen presentation.

Additionally, oncolytic viruses can be engineered to express proinflammatory cytokines such as IL-12 or IL-4 (R. J. Wong et al. 2004; Post et al. 2007), chemokines such as CCL5 or CCL3 (Li et al. 2011) or costimulatory molecules such as CD40 ligand (Galivo et al. 2010). Special cases are oncolytic viruses expressing tumor associated antigens. These viruses effectively act as oncolytic vaccines (Bridle et al. 2010).

One of the major obstacles of oncolytic virotherapy is the premature clearance of the virus by the immune system. This is an important setback for the development of systemic delivery of viral therapeutics. Rapid viral clearance by pre-existing antibodies can be circumvented when using other serotypes of the particular virus. However, this only works for viruses against whom immune system develops antibodies that don't exhibit cross-immunity against other serotypes (adenoviruses or vesicular stomatitis virus) and viral particles still face other obstacles in biodistribution. Therefore, more sophisticated methods have to be employed.

Coating the surface of viral particles in polymers, liposomes, nanoparticles, calcium residues and, most notably, PEG (polyethylene glycol), had been shown to reduce sequestration in the liver and to increase systemic toxicity of the virus (J. Chen et al. 2016; Tesfay et al. 2013). A method which would use infected cells as systemic carriers is also being researched. Autologous cells (for example mesenchymal stem cells or dendritic cells) can be infected *ex vivo* and then reinfused back to the patient, functioning as cell carriers (Mader et al. 2009).

Even if virus successfully infects a tumor site, its spread is often impaired by tumor size and heterogenicity. Therefore, some oncolytic viruses were engineered to express ECM-degrading enzymes, such as hyaluronidase (Guedan et al. 2010). Others were engineered to express fusogenic membrane proteins, infecting neighbouring cells by creating syncitia rather than spreading through the tumor microenvironment (Ebert et al. 2004).

Oncolytic viruses can also be creatively utilized for cancer imaging. The field is currently in a dire need of a non-invasive assessment of biodistribution of oncolytic viruses and, most importantly, a way of detecting tumor origin and metastases. Thanks to its large 192 kbp long genome, vaccinia virus was recently utilized for such purpose (Haddad 2017). Vaccinia virus can be equiped with the so-called reporter genes which can make such specific non-invasive method of cancer imaging possible. The reporter genes include GFP, human norepinephrine transporter, human sodium-iodide transporter, Renilla luciferase or human somatostatin receptor 2 (N. Chen and Zhang 2009; McCart et al. 2004). An example of molecular imaging utilizing genetically modified vaccinia virus is shown in Figure 3.

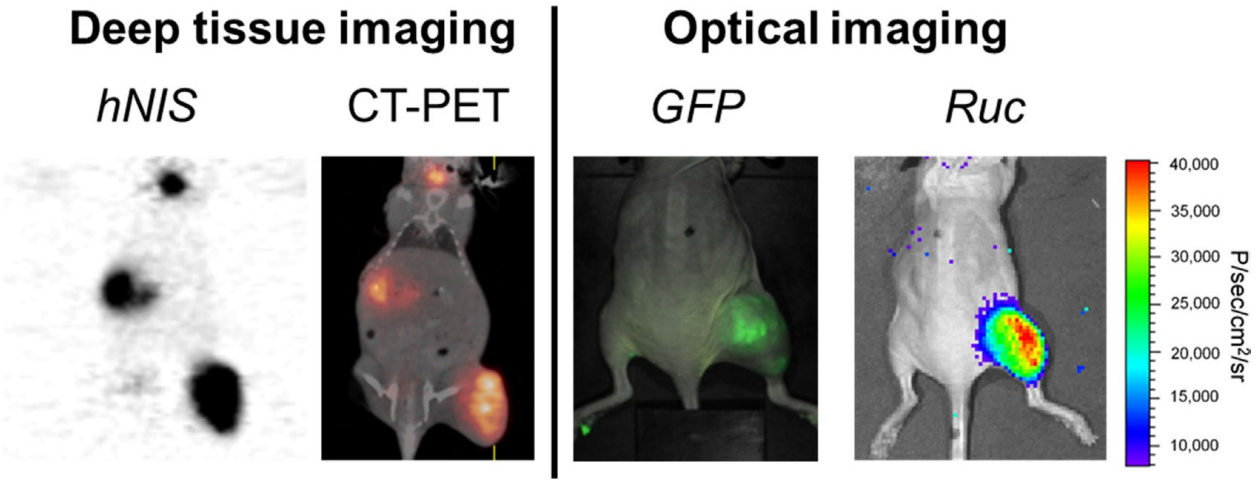

Figure 3: Deep tissue and optical imaging of tumors utlizing GLV-1h153 construct of vaccinia virus in mice bearing PANC-1 xenografts (Haddad et al. 2012)**.** The virus was modified to express GFP, human sodium iodide symporter (hNIS) and Ruc genes. A combination of imaging techniques can show the sites of the tumor and metastases, which had been infected by the said virus, thus allowing for a precise and non-invasive way of tumor detection. *CT-PET*- computed tomography-positron emission tomography, *GFP* – green fluorescent protein, *hNIS* – human sodium iodide symporter, *P* – photons or photon flux, *PANC-1* – a human pancreatic cancer cell line, *sr* – steradian, *RLuc* – Renilla luciferase. Image adopted from (Haddad 2017).

2.3 Oncolytic viruses in clinical development

Viruses are incredibly diverse in their characteristics, tropism, biodistribution, virulence and many other factors. Similarly, due to their high mutational burden, human tumors are genetically variable even to the point at which each one of the tumors can be considered unique. It is therefore unlikely that one oncolytic virus would become the best candidate for general cancer treatment, and so it is reasonable to develop additional oncolytic virotherapeutics from a variety of virus strains (Russell and Peng 2017). Moreover, a strong curative potential may lie in a therapy utilizing the combination of oncolytic viruses. As of today, a variety of oncolytic viruses is being researched and evaluated in clinical trials. An introduction of some of the most prominent types of oncolytic viruses can be found in this chapter. A brief overview of some examples of the ongoing or recently conducted trials can be found in Table 2 at the end of the chapter.

2.3.1 Herpes simplex virus

Herpes simplex viruses, and particularly HSV-1, are one of the most widespread kinds of viruses currently researched as potential virotherapeutics. T-VEC, first oncolytic virus approved for cancer treatment in the US and Europe, is an HSV-1 virus. As Russell and Peng state in their review: "... T-VEC is an ideal intratumoral cancer vaccine. It spreads locally within the injected tumor and kills tumor cells by in situ necroptosis, causing them to release tumor antigens, viral antigens, damage-associated molecular patterns (DAMPs), and GM-CSF, providing what is possibly a near-perfect environment for activated APCs to phagocytose a mixture of viral and tumor antigens for presentation to $CD4^+$ helper and $CD8^+$ cytotoxic T cells in the regional lymph nodes." (Russell and Peng 2017).

HSV-M032, engineered to overexpress IL-12 in tumor sites, enters a Phase I clinical trial to treat patients suffering from glioblastoma (Patel et al. 2016). HSV-G207 is attenuated by deletion of the ICP34.5 region and by mutation of the ICP6 gene, which impair its neurovirulence in normal cells. It is a promising candidate for the treatment of brain tumors and it enters Phase I clinical trial (Waters et al. 2017).

2.3.2 Adenovirus

ONYX-15 is an adenovirus targeted to cause lysis of cells with defective p53 pathway. It was approved for cancer treatment in China in 2006 (Garber 2006), but its development in the US and Europe was stopped due to the unconvincing results.

Ad-CG0070 is a genetically modified adenovirus expressing GM-CSF and targeted against cells with an aberrant Rb-pathway (Ramesh et al. 2006). It was evaluated in Phase I trial for the treatment of bladder cancer (Burke et al. 2012), showing tolerable safety profile and oncolytic activity.

ONCOS-102 is a GM-CSF expressing adenovirus. In 2018, it entered Phase I/II clinical trial focused on evaluating the safety of combination of ONCOS-102 with DC vaccine in men with metastatic prostate cancer. The trial is conducted by the Urology Clinic of the University Hospital in Motol, Prague (NCT03514836).

2.3.3 Vaccinia virus

A vaccinia virus based GL-ONC1 was evaluated for treatment of patients suffering from head and neck cancer in combination with radio- and chemotherapy, showing sufficient safety profile (Mell et al. 2017).

JX-594 (Pexa-Vec) is a highly promising TK- vaccinia virus based oncolytic vector engineered to express GM-CSF. JX-594 was evaluated in a Phase II clinical trial against hepatocellular carcinoma, in which immune responses were observed in injected but also in uninjected distant tumors (Heo et al. 2013).

2.3.4 Coxsackievirus

Coxsackievirus is an RNA virus belonging to the family Picornaviridae.

CVA21 is an oncolytic Coxsackievirus targeting DAF and ICAM-1 complex as its receptor (D. R. Shafren et al. 1997). Expression of both DAF and ICAM-1 is upregulated in various cancers, including melanoma (Cheung et al. 1988). CVA21 had been used in a Phase II clinical trial to treat advanced melanoma (Andtbacka et al. 2015). The trial proved its efficacy and safety, as the therapy didn't induce any serious adverse effects.

2.3.5 Newcastle disease virus

NDV is an avian virus non-pathogenic for humans, with proven oncolytic and immunostimulatory effects in human patients.

In a Phase I trial NDV PV701 was tested for the treatment of solid tumors unresponsive to chemotherapy (Pecora et al. 2002). PV701 caused no severe adverse effects, but responses in tumor beds were observed. Additionally, electron microscopy of a tumor sample taken 11 months after the therapy found active virus particles budding from the tumor cells.

2.3.6 Reovirus

Probably the most prominent of the oncolytic reoviruses is Reolysin, which has already been a subject of several studies. In one of the most recent clinical trials, Reolysin was tested in combination with chemotherapy for treatment of breast cancer. It was reported that Reolysin caused a statistically significant improvement in overall survival (Bernstein et al. 2017). In a Phase II clinical study Reolysin plus chemotherapy was evaluated in treatment of NSCLC, with 89 % of the treated patients experiencing clinical benefit (Villalona-Calero et al. 2016).

It has to be noted though, that the responses of the improvement in OS tend to be delayed, as Reolysin acts mainly as an immunostimulatory agent rather than a cytolytic one (Fountzilas, Patel, and Mahalingam 2017).

Moreover, reovirus might be a promising candidate for the treatment of brain tumors. In a recent study, an oncolytic reovirus was shown to be able to cross the blood-brain barrier after

intravenous administration and to successfully infect brain tumors of the treated patients (Errington-Mais et al. 2018).

2.3.7 Measles virus

Measles virus propagates between cells by fusing with membranes of neighbouring cells and creating syncitia. This could prove beneficial for its biodistribution in the tumor microenvironment.

MV-NIS, a measles virus expressing human sodium-iodide symporter, was evaluated in a Phase I study as a treatment of ovarian cancer non-responsive for chemotherapy. The treatment caused no severe adverse effects and the induction of anti-tumor immune response was reported. The accumulation of virus in tumor bed was confirmed thanks to functional imaging (Galanis et al. 2015).

2.3.8 Other viruses

A number of oncolytic viruses in clinical development and many recent clinical trials have been omitted in this chapter, as this work focuses on the general principles of oncolytic virotherapy. To present at least a hint of the full picture, it has to be mentioned that parvoviruses (Geletneky et al. 2014), poliovirus (Goetz et al. 2011), Seneca Valley virus (Rudin et al. 2011) and even retroviruses (Cloughesy et al. 2016) are being researched for their oncolytic activity. Parvoviruses are especially interesting for the treatment of brain tumors, as they proved capable of crossing the blood-brain barrier (Hajda et al. 2014).

Table 2: Examples of some recent trials evaluating the safety and efficacy of oncolytic virotherapies.
NSCLC – non-small cell lung carcinoma.

Virus type	Name	Indication	Stage	Reference
Herpes simplex	HSV-M032	Glioblastoma	Phase I	(Patel et al. 2016)
	HSV-G207	Brain tumors	Phase I	(Waters et al. 2017)

Adenovirus	Ad-CG0070	Bladder cancer	Phase I	(Burke et al. 2012)
	ONCOS-102	Prostate cancer	Phase I/II	(NCT03514836 2018)
Vaccinia virus	GL-ONC1	Head and neck cancer	Phase I	(Mell et al. 2017)
	JX-594	Hepatocellular carcinoma	Phase II	(Heo et al. 2013)
Coxsackievirus	CVA21	Melanoma	Phase II	(Andtbacka et al. 2015)
Reovirus	Reolysin®	NSCLC	Phase II	(Villalona-Calero et al. 2016)
		Breast cancer	Phase II	(Bernstein et al. 2017)
Measles virus	MV-NIS	Ovarian cancer	Phase I	(Galanis et al. 2015)

2.4 Oncolytic viruses in combination with other cancer treatments

Despite their oncolytic potential, oncolytic viruses were rarely shown to cause a long-term regression of the disease by themselves. It is therefore almost certain, that in clinical practice, oncolytic virotherapy will be used in tandem with other cancer treatments. A number of studies researching the efficacy of oncolytic viruses in combination with other agents have already been done, this chapter aims to introduce some of them and the potential approaches of combinational therapy.

Cyclophosphamide (CPA) is an immunosuppressant shown to reduce the number of innate immune cells and T_{reg} cells in the patient and thus partially dampening the effects of immunosuppressive cells in tumor microenvironment (Thomas et al. 2008). Moreover, it lowers the levels of antibodies in patient's blood. Cyclophosphamide was therefore utilized in combination with oncolytic viruses in hopes of stronger induction of anti-tumor immune response. Indeed, in a study employing vaccinia virus and cyclophosphamide in an animal model, stronger anti-tumor immune response and better viral distribution in tumors were reported (Hofmann, Weibel, and Szalay 2014).

Histone deacetylase inhibitors are capable of dampening the antiviral immune response and thus improving the spread of the virus (Alvarez-Breckenridge, Kaur, and Chiocca 2009).

To increase the rate of crossing of viral particles from blood vessels into tumor beds and so improving viral delivery, a number of studies employing vasoactive agents have been made. The agents in question were, for example, histamine, nitroglycerin (Bilbao et al. 2000) or local hyperthermia (Eisenberg et al. 2010).

Another obstacle of viral spread is the size of the tumor and especially the extracellular matrix (ECM) slowing the spread of viral particles between tumor cells. Hyaluronidase or collagenase, ECM-degrading enzymes, were used for pre-degradation of tumor ECM in hopes of increasing viral distribution (Ganesh et al. 2008).

Tyrosine-kinase inhibitor sunitinib is an antiangiogenic agent which can increase anti-tumor immunity (Bose et al. 2011). It was also shown to increase the rate of replication of vesicular stomatitis virus (Jha et al. 2013). Sorafenib, another tyrosine-kinase inhibitor, showed synergistic effects with Pexa-Vec when used in combinational tumor treatment (Heo et al. 2011).

The most reasonable combination is of course the tandem of oncolytic viruses with already established cancer therapies. Therefore, a number of studies were made to evaluate the safety profile and efficacy of oncolytic viruses in combination with surgery, chemotherapy and radiotherapy (Lolkema et al. 2011; Mell et al. 2017). Vaccinia virus showed synergistic effects and increase in median survival when used with radiotherapy (Wilkinson et al. 2016).

Another approach is to combine oncolytic viruses with cellular therapies. For example, a DC vaccine was combined with oncolytic vaccinia virus expressing T cell attracting chemokines (Li et al. 2011). Oncolytic virotherapy had also been evaluated in combination with adoptive T cell therapy utilizing transgenic TAA-specific T cells (Rommelfanger et al. 2012). Vaccinia virus was also combined with CAR T cells with synergistic effects in a mouse model of solid tumor (Nishio et al. 2014).

Another logical approach is to combine oncolytic viruses with the rising star of cancer immunotherapy - immune checkpoint inhibitors. Vaccinia virus showed improved efficacy when combined with CTLA-4 inhibitors, but the timing of the administration of CTLA-4 inhibitors was important (Haddad 2017). If a dose of oncolytic virus was injected on the same day that immune checkpoint inhibitor therapy started, the oncolytic effect of virus was

dampened. The best results were achieved when administrating CTLA-4 inhibitors three days after the start of oncolytic virotherapy.

Tumor cells overexpressing PD-L1 are more susceptible to treatment with immune checkpoint inhibitors (Gordon et al. 2014). Oncolytic viruses induce expression of IFN, which in turn upregulates expression of PD-L1 (Bellucci et al. 2015). Oncolytic virotherapy could therefore prime for immune checkpoint blockade therapy, as was shown in a case of reovirus against brain tumors (Errington-Mais et al. 2018). CVA21 and pembrolizumab were combined to treat melanoma (D. Shafren et al. 2017) and Reolysin plus PD1 inhibitors prolonged the overall survival of mice suffering from melanoma (Rajani et al. 2016). Moreover, oncolytic viruses can be engineered to express antibodies against immune checkpoint receptors, which led to enhanced anti-tumor activity in case of measles virus (Engeland et al. 2014).

Finally, some oncolytic viruses could be combined with other oncolytic viruses. Vesicular stomatitis virus in combination with vaccinia virus increased the median survival in a mouse model (Le Boeuf et al. 2010). Using two different oncolytic viruses in a subsequent fashion could also circumvent the problem of immunodominant viral antigens. A therapy with vesicular stomatitis virus was more effective if the host's immune system had first been primed with an adenovirus expressing TAAs, thus increasing the anti-tumor immune response at the expense of anti-viral immune response (Bramson et al. 2013).

While combinational therapy holds a great potential, it also brings a number of challenges. For example, oncolytic viruses prefer to replicate in dividing cells, but chemotherapy kills most proliferating cells. The leaky vasculature improves the viral distribution to the tumor and anti-angiogenic therapy could therefore be antagonistic. While low dose CPA treatment depletes T_{reg} cells and increases anti-tumor immune response, it also promotes anti-viral immune response. The administration of high doses of CPA on the other hand, causes widespread immune depletion and enhances viral spread, but dampens anti-tumor immunity. The usage of immune checkpoint inhibitors in combinational therapy could unleash anti-viral response responsible for premature viral clearance (Chiocca and Rabkin 2014).

Nevertheless, the studies strongly suggest that combining oncolytic viruses with other cancer treatments is the future of oncolytic virotherapy.

Conclusion

Oncolytic virotherapy has made a long journey in the past seventy years. Despite the often-disappointing results of the trials in the 20th century, the approval of T-VEC proved that oncolytic viruses have a real potential to become a cancer treatment in clinical practice. Moreover, it seems that a number of new oncolytic viruses are soon to be approved for clinical use. In particular, JX594 (Pexa-Vec) for the treatment of hepatocellular carcinoma, adenovirus CG0070 for the treatment of bladder cancer, Reolysin as an agent against head and neck cancer and HSV-1 G47δ for glioblastoma treatment (Lundstrom 2018). The field of oncolytic virotherapy still faces many challenges. First of all, viruses need to be further adapted to improve their efficacy and specific targeting of cancer cells. Secondly, researchers have to evaluate the myriad of possible combinations of oncolytic viruses with other cancer therapies to successfully determine the right treatment for patients suffering from various types of cancer.

But despite all the obstacles, the recent uplifting results of oncolytic virotherapy could start a new era of cancer treatment. And, alongside other immunotherapies, oncolytic viruses could enrich the current therapeutic portfolio of anti-cancer drugs and strategies with the hope to find the optimal regimen which will cure the cancer in the near future.

References

Alvarez-Breckenridge, Christopher, Balveen Kaur, and E. Antonio Chiocca. 2009. “Pharmacologic and Chemical Adjuvants in Tumor Virotherapy.” *Chemical Reviews* 109 (7): 3125–40. https://doi.org/10.1021/cr900048k.

Amiri-Kordestani, L., G. M. Blumenthal, Q. C. Xu, L. Zhang, S. W. Tang, L. Ha, W. C. Weinberg, et al. 2014. “FDA Approval: Ado-Trastuzumab Emtansine for the Treatment of Patients with HER2-Positive Metastatic Breast Cancer.” *Clinical Cancer Research* 20 (17): 4436–41. https://doi.org/10.1158/1078-0432.CCR-14-0012.

Andtbacka, R H I, Brendan D Curti, Howard Kaufmann, Gregory A Daniels, John J Nemunaitis, Lynn E Spitler, Sigrun Hallmeyer, et al. 2015. “Final Data from CALM: A Phase II Study of Coxsackievirus A21 (CVA21) Oncolytic Virus Immunotherapy in Patients with Advanced Melanoma.” *J Clin Oncol* 33: (suppl;abstr9030).

Bellucci, Roberto, Allison Martin, Davide Bommarito, Kathy Wang, Steen H Hansen, Gordon J Freeman, and Jerome Ritz. 2015. “Interferon-γ-Induced Activation of JAK1 and JAK2 Suppresses Tumor Cell Susceptibility to NK Cells through Upregulation of PD-L1 Expression.” *OncoImmunology* 4 (6): e1008824. https://doi.org/10.1080/2162402X.2015.1008824.

Bernstein, Vanessa, Susan Ellard, Susan F. Dent, Karen A. Gelmon, Sukhbinder K. Dhesy-Thind, Mihaela Mates, Muhammed Salim, et al. 2017. “Abstract CT131: A Randomized (RCT) Phase II Study of Oncolytic Reovirus (Pelareorep) plus Standard Weekly Paclitaxel (P) as Therapy for Metastatic Breast Cancer (MBC).” *Cancer Research* 77 (13 Supplement): CT131–CT131. https://doi.org/10.1158/1538-7445.AM2017-CT131.

Beusechem, Victor W. Van, Petra B. Van den Doel, Jacques Grill, Herbert M. Pinedo, and Winald R. Gerritsen. 2002. “Conditionally Replicative Adenovirus Expressing P53 Exhibits Enhanced Oncolytic Potency.” *Cancer Research* 62 (21): 6165–71.

Bilbao, R, M Bustos, P Alzuguren, M J Pajares, M Drozdzik, C Qian, and J Prieto. 2000. “A Blood–Tumor Barrier Limits Gene Transfer to Experimental Liver Cancer: The Effect of Vasoactive Compounds.” *Gene Therapy* 7 (21): 1824–32. https://doi.org/10.1038/sj.gt.3301312.

Boeuf, Fabrice Le, Jean-Simon Diallo, J Andrea McCart, Steve Thorne, Theresa Falls, Marianne Stanford, Femina Kanji, et al. 2010. “Synergistic Interaction Between Oncolytic Viruses Augments Tumor Killing.” *Molecular Therapy* 18 (5): 888–95. https://doi.org/10.1038/MT.2010.44.

Bose, Anamika, Jennifer L. Taylor, Sean Alber, Simon C. Watkins, Jorge A. Garcia, Brian I. Rini, Jennifer S. Ko, Peter A. Cohen, James H. Finke, and Walter J. Storkus. 2011. “Sunitinib Facilitates the Activation and Recruitment of Therapeutic Anti-Tumor Immunity in Concert with Specific Vaccination.” *International Journal of Cancer* 129 (9): 2158–70. https://doi.org/10.1002/ijc.25863.

Bramson, Jonathan L, Liang Zhang, Yonghong Wan, Jonathan Pol, Derek Clouthier, Byram W Bridle, Brian D Lichty, and Lan Chen. 2013. “ Oncolytic Vesicular Stomatitis Virus Quantitatively and Qualitatively Improves Primary CD8 + T-Cell Responses to Anticancer Vaccines .” *OncoImmunology* 2 (8): e26013. https://doi.org/10.4161/onci.26013.

Breitbach, C. J., R. Arulanandam, N. De Silva, S. H. Thorne, R. Patt, M. Daneshmand, A. Moon, et al. 2013. “Oncolytic Vaccinia Virus Disrupts Tumor-Associated Vasculature in Humans.” *Cancer Research* 73 (4):

1265–75. https://doi.org/10.1158/0008-5472.CAN-12-2687.

Breitbach, Caroline J, Naomi S De Silva, Theresa J Falls, Usaf Aladl, Laura Evgin, Jennifer Paterson, Yang Yang Sun, et al. 2011. “Targeting Tumor Vasculature With an Oncolytic Virus.” *Molecular Therapy* 19 (5): 886–94. https://doi.org/10.1038/MT.2011.26.

Bridle, Byram W, Kyle B Stephenson, Jeanette E Boudreau, Sandeep Koshy, Natasha Kazdhan, Eleanor Pullenayegum, Jérôme Brunellière, Jonathan L Bramson, Brian D Lichty, and Yonghong Wan. 2010. “Potentiating Cancer Immunotherapy Using an Oncolytic Virus.” *Molecular Therapy* 18 (8): 1430–39. https://doi.org/10.1038/MT.2010.98.

Buller, R. M. L., G. L. Smith, K. Cremer, A. L. Notkins, and Bernard Moss. 1985. “Decreased Virulence of Recombinant Vaccinia Virus Expression Vectors Is Associated with a Thymidine Kinase-Negative Phenotype.” *Nature* 317 (6040): 813–15. https://doi.org/10.1038/317813a0.

Burke, James M., Donald L. Lamm, Maxwell V. Meng, John J. Nemunaitis, Joseph J. Stephenson, James C. Arseneau, Junko Aimi, et al. 2012. “A First in Human Phase 1 Study of CG0070, a GM-CSF Expressing Oncolytic Adenovirus, for the Treatment of Nonmuscle Invasive Bladder Cancer.” *Journal of Urology* 188 (6): 2391–97. https://doi.org/10.1016/j.juro.2012.07.097.

Cassady, Kevin A., Kellie B. Haworth, Josh Jackson, James M. Markert, and Timothy P. Cripe. 2016. “To Infection and beyond: The Multi-Pronged Anti-Cancer Mechanisms of Oncolytic Viruses.” *Viruses* 8 (2): 1–9. https://doi.org/10.3390/v8020043.

Chang, Jianhua, Xiaoying Zhao, Xianghua Wu, Ye Guo, Haiyi Guo, Junnin Cao, Yong Guo, Delun Lou, Dechao Yu, and Jin Li. 2009. “A Phase I Study of KH901, a Conditionally Replicating Granulocyte-Macrophage Colony-Stimulating Factor: Armed Oncolytic Adenovirus for the Treatment of Head and Neck Cancers.” *Cancer Biology & Therapy* 8 (8): 676–82. https://doi.org/10.4161/cbt.8.8.7913.

Chen, Jianhua, Pei Gao, Sujing Yuan, Rongxin Li, Aimin Ni, Liang Chu, Li Ding, Ying Sun, Xin-Yuan Liu, and Yourong Duan. 2016. “Oncolytic Adenovirus Complexes Coated with Lipids and Calcium Phosphate for Cancer Gene Therapy.” *ACS Nano* 10 (12): 11548–60. https://doi.org/10.1021/acsnano.6b06182.

Chen, Nanhai, and Qian Zhang. 2009. “A Novel Recombinant Vaccinia Virus Expressing the Human Norepinephrine Transporter Retains Oncolytic Potential and Facilitates Deep-Tissue Imaging.” *Molecular Medicine* 15 (5–6): 1. https://doi.org/10.2119/molmed.2009.00014.

Chen, Y, T DeWeese, J Dilley, Y Zhang, Y Li, N Ramesh, J Lee, et al. 2001. “CV706, a Prostate Cancer-Specific Adenovirus Variant, in Combination with Radiotherapy Produces Synergistic Antitumor Efficacy without Increasing Toxicity.” *Cancer Research* 61 (14): 5453–60. http://www.ncbi.nlm.nih.gov/pubmed/11454691.

Cheung, N K, E I Walter, W H Smith-Mensah, W D Ratnoff, M L Tykocinski, and M E Medof. 1988. “Decay-Accelerating Factor Protects Human Tumor Cells from Complement-Mediated Cytotoxicity in Vitro.” *The Journal of Clinical Investigation* 81 (4): 1122–28. https://doi.org/10.1172/JCI113426.

Chiocca, E. A., and S. D. Rabkin. 2014. “Oncolytic Viruses and Their Application to Cancer Immunotherapy.” *Cancer Immunology Research* 2 (4): 295–300. https://doi.org/10.1158/2326-6066.cir-14-0015.

Clemens, Michael J. 2004. “Targets and Mechanisms for the Regulation of Translation in Malignant Transformation.” *Oncogene* 23 (18): 3180–88. https://doi.org/10.1038/sj.onc.1207544.

Cloughesy, Timothy F, Joseph Landolfi, Daniel J Hogan, Stephen Bloomfield, Bob Carter, Clark C Chen, J Bradley Elder, et al. 2016. “Phase 1 Trial of Vocimagene Amiretrorepvec and 5-Fluorocytosine for Recurrent High-Grade Glioma.” *Science Translational Medicine* 8 (341): 341ra75.

https://doi.org/10.1126/scitranslmed.aad9784.

Doronin, K, K Toth, M Kuppuswamy, P Ward, A E Tollefson, and W S Wold. 2000. “Tumor-Specific, Replication-Competent Adenovirus Vectors Overexpressing the Adenovirus Death Protein.” *Journal of Virology* 74 (13): 6147–55. https://doi.org/10.1128/JVI.74.13.6147-6155.2000.

Ebert, Oliver, Katsunori Shinozaki, Chryssanthi Kournioti, Man-Seong Park, Adolfo García-Sastre, and Savio L. C. Woo. 2004. “Syncytia Induction Enhances the Oncolytic Potential of Vesicular Stomatitis Virus in Virotherapy for Cancer.” *Cancer Research* 64 (9): 3265–70. https://doi.org/10.1158/0008-5472.CAN-03-3753.

Eisenberg, David P., Susanne G. Carpenter, Prasad S. Adusumilli, Mei-Ki Chan, Karen J. Hendershott, Zhenkun Yu, and Yuman Fong. 2010. “Hyperthermia Potentiates Oncolytic Herpes Viral Killing of Pancreatic Cancer through a Heat Shock Protein Pathway.” *Surgery* 148 (2): 325–34. https://doi.org/10.1016/J.SURG.2010.05.005.

Engeland, Christine E, Christian Grossardt, Rūta Veinalde, Sascha Bossow, Diana Lutz, Johanna K Kaufmann, Ivan Shevchenko, et al. 2014. “CTLA-4 and PD-L1 Checkpoint Blockade Enhances Oncolytic Measles Virus Therapy.” *Molecular Therapy* 22 (11): 1949–59. https://doi.org/10.1038/MT.2014.160.

Errington-Mais, Fiona, Julia V. Cockle, Andrew J. Furness, Fiona J. Collinson, Hardev Pandha, Stephen D. Griffin, Elizabeth J. Ilett, et al. 2018. *Intravenous Delivery of Oncolytic Reovirus to Brain Tumor Patients Immunologically Primes for Subsequent Checkpoint Blockade*. *Science Translational Medicine*. Vol. 10. https://doi.org/10.1126/scitranslmed.aam7577.

Fan, Gaowei, Zujian Wang, Mingju Hao, and Jinming Li. 2015. “Bispecific Antibodies and Their Applications.” *Journal of Hematology & Oncology* 8 (1): 130. https://doi.org/10.1186/s13045-015-0227-0.

Fenoglio, Daniela, Paolo Traverso, Alessia Parodi, Francesca Kalli, Maurizio Zanetti, and Gilberto Filaci. 2013. “Generation of More Effective Cancer Vaccines.” *Human Vaccines & Immunotherapeutics* 9 (12): 2543–47. https://doi.org/10.4161/hv.26147.

Fountzilas, Christos, Sukeshi Patel, and Devalingam Mahalingam. 2017. “Review: Oncolytic Virotherapy, Updates and Future Directions.” *Oncotarget* 8 (60): 102617–39. https://doi.org/10.18632/oncotarget.18309.

Freytag, Svend O, Hans Stricker, Jan Pegg, Dell Paielli, Deepak G Pradhan, James Peabody, Mariza DePeralta-Venturina, et al. 2003. “Phase I Study of Replication-Competent Adenovirus-Mediated Double-Suicide Gene Therapy in Combination with Conventional-Dose Three-Dimensional Conformal Radiation Therapy for the Treatment of Newly Diagnosed, Intermediate- to High-Risk Prostate Cancer.” *Cancer Research* 63 (21): 7497–7506. http://www.ncbi.nlm.nih.gov/pubmed/14612551.

Galanis, E., P. J. Atherton, M. J. Maurer, K. L. Knutson, S. C. Dowdy, W. A. Cliby, P. Haluska, et al. 2015. “Oncolytic Measles Virus Expressing the Sodium Iodide Symporter to Treat Drug-Resistant Ovarian Cancer.” *Cancer Research* 75 (1): 22–30. https://doi.org/10.1158/0008-5472.CAN-14-2533.

Galivo, Feorillo, Rosa Maria Diaz, Uma Thanarajasingam, Dragan Jevremovic, Phonphimon Wongthida, Jill Thompson, Timothy Kottke, Glen N. Barber, Alan Melcher, and Richard G. Vile. 2010. “Interference of CD40L-Mediated Tumor Immunotherapy by Oncolytic Vesicular Stomatitis Virus.” *Human Gene Therapy* 21 (4): 439–50. https://doi.org/10.1089/hum.2009.143.

Ganesh, S., M. Gonzalez-Edick, D. Gibbons, M. Van Roey, and K. Jooss. 2008. “Intratumoral Coadministration of Hyaluronidase Enzyme and Oncolytic Adenoviruses Enhances Virus Potency in Metastatic Tumor Models.” *Clinical Cancer Research* 14 (12): 3933–41. https://doi.org/10.1158/1078-0432.CCR-07-4732.

Garber, K. 2006. “China Approves World’s First Oncolytic Virus Therapy for Cancer Treatment.” *Journal of the National Cancer Institute* 98 (5): 298–300. https://doi.org/10.1093/jnci/djj111.

Gardner, Thomas, Bennett Elzey, and Noah M. Hahn. 2012. “Sipuleucel-T (Provenge) Autologous Vaccine Approved for Treatment of Men with Asymptomatic or Minimally Symptomatic Castrate-Resistant Metastatic Prostate Cancer.” *Human Vaccines & Immunotherapeutics* 8 (4): 534–39. https://doi.org/10.4161/hv.19795.

Geletneky, Karsten, Johannes Huesing, Michael Dahm, Ottheinz Krebs, Bernard Huber, David Capper, Jean Rommelaere, Jacek Hajda, and Andreas Unterberg. 2014. “First Combined Intravenous and Intracerebral Application of an Oncolytic Virus, Parvovirus h-1, in a Phase I/IIa Clinical Trial in Patients with Recurrent Glioblastoma Multiforme (ParvOryx01).” *Journal of Clinical Oncology* 32 (15_suppl): TPS2111–TPS2111. https://doi.org/10.1200/jco.2014.32.15_suppl.tps2111.

Gholami, Sepideh, Andrew Marano, Nanhai G. Chen, Richard J. Aguilar, Alexa Frentzen, Chun-Hao Chen, Emil Lou, et al. 2014. “A Novel Vaccinia Virus with Dual Oncolytic and Anti-Angiogenic Therapeutic Effects against Triple-Negative Breast Cancer.” *Breast Cancer Research and Treatment* 148 (3): 489–99. https://doi.org/10.1007/s10549-014-3180-7.

Gilham, David Edward, John Anderson, John Stephen Bridgeman, Robert Edward Hawkins, Mark Adrian Exley, Hans Stauss, John Maher, et al. 2015. “Adoptive T-Cell Therapy for Cancer in the United Kingdom: A Review of Activity for the British Society of Gene and Cell Therapy Annual Meeting 2015.” *Human Gene Therapy* 26 (5): 276–85. https://doi.org/10.1089/hum.2015.024.

Goetz, Christian, Elena Dobrikova, Mayya Shveygert, Mikhail Dobrikov, and Matthias Gromeier. 2011. “Oncolytic Poliovirus against Malignant Glioma.” *Future Virology* 6 (9): 1045–58. https://doi.org/10.2217/fvl.11.76.

Gordon, Michael S., Ira Mellman, Maya Leabman, Jeffery A. Sosman, Roy S. Herbst, Ahmad Mokatrin, Omid Hamid, et al. 2014. “Predictive Correlates of Response to the Anti-PD-L1 Antibody MPDL3280A in Cancer Patients.” *Nature* 515 (7528): 563–67. https://doi.org/10.1038/nature14011.

Govers, Coen, Zsolt Sebestyén, Miriam Coccoris, Ralph A. Willemsen, and Reno Debets. 2010. “T Cell Receptor Gene Therapy: Strategies for Optimizing Transgenic TCR Pairing.” *Trends in Molecular Medicine* 16 (2): 77–87. https://doi.org/10.1016/J.MOLMED.2009.12.004.

Grillo-Lopez, A., C. White, B. Dallaire, C. Varns, C. Shen, A. Wei, J. Leonard, et al. 2000. “Rituximab The First Monoclonal Antibody Approved for the Treatment of Lymphoma.” *Current Pharmaceutical Biotechnology* 1 (1): 1–9. https://doi.org/10.2174/1389201003379059.

Guedan, Sonia, Juan José Rojas, Alena Gros, Elena Mercade, Manel Cascallo, and Ramon Alemany. 2010. “Hyaluronidase Expression by an Oncolytic Adenovirus Enhances Its Intratumoral Spread and Suppresses Tumor Growth.” *Molecular Therapy* 18 (7): 1275–83. https://doi.org/10.1038/MT.2010.79.

Guo, Chunqing, Masoud H Manjili, John R Subjeck, Devanand Sarkar, Paul B Fisher, and Xiang-Yang Wang. 2013. “Therapeutic Cancer Vaccines: Past, Present, and Future.” *Advances in Cancer Research* 119: 421–75. https://doi.org/10.1016/B978-0-12-407190-2.00007-1.

Guse, Kilian, Vincenzo Cerullo, and Akseli Hemminki. 2011. “Oncolytic Vaccinia Virus for the Treatment of Cancer.” *Expert Opinion on Biological Therapy* 11 (5): 595–608. https://doi.org/10.1517/14712598.2011.558838.

Haddad, Dana. 2017. “Genetically Engineered Vaccinia Viruses As Agents for Cancer Treatment, Imaging, and Transgene Delivery.” *Frontiers in Oncology* 7 (May): 1–12. https://doi.org/10.3389/fonc.2017.00096.

Haddad, Dana, Chun-Hao Chen, Sean Carlin, Gerd Silberhumer, Nanhai G. Chen, Qian Zhang, Valerie Longo, et al. 2012. “Imaging Characteristics, Tissue Distribution, and Spread of a Novel Oncolytic Vaccinia Virus Carrying the Human Sodium Iodide Symporter.” Edited by Juri G. Gelovani. *PLoS ONE* 7 (8): e41647. https://doi.org/10.1371/journal.pone.0041647.

Hajda, J., O. Krebs, D. Capper, B. Huber, K. Geletneky, A. Angelova, B. Leuchs, et al. 2014. “Et-21 * Combination of Intravenous and Intracerebral Injection of Oncolytic Parvovirus H-1 in a Phase I/Iia Clinical Trial of Patients With Recurrent Glioblastoma Multiforme: Penetration of H-1 Virus Across the Blood-Brain Barrier.” *Neuro-Oncology* 16 (suppl 5): v83–84. https://doi.org/10.1093/neuonc/nou255.21.

Hammond, A L, R K Plemper, J Zhang, U Schneider, S J Russell, and R Cattaneo. 2001. “Single-Chain Antibody Displayed on a Recombinant Measles Virus Confers Entry through the Tumor-Associated Carcinoembryonic Antigen.” *Journal of Virology* 75 (5): 2087–96. https://doi.org/10.1128/JVI.75.5.2087-2096.2001.

Hanahan, Douglas, and Robert A. Weinberg. 2011. “Hallmarks of Cancer: The Next Generation.” *Cell* 144 (5): 646–74. https://doi.org/10.1016/J.CELL.2011.02.013.

Heise, Carla, Adam Sampson-Johannes, Angelica Williams, Frank Mccormick, Daniel D. Von Hoff, and David H. Kirn. 1997. “ONYX-015, an E1B Gene-Attenuated Adenovirus, Causes Tumor-Specific Cytolysis and Antitumoral Efficacy That Can Be Augmented by Standard Chemotherapeutic Agents.” *Nature Medicine* 3 (6): 639–45. https://doi.org/10.1038/nm0697-639.

Heo, Jeong, Caroline J Breitbach, Anne Moon, Chang Won Kim, Rick Patt, Mi Kyung Kim, Yu Kyung Lee, et al. 2011. “Sequential Therapy With JX-594, A Targeted Oncolytic Poxvirus, Followed by Sorafenib in Hepatocellular Carcinoma: Preclinical and Clinical Demonstration of Combination Efficacy.” *Molecular Therapy* 19 (6): 1170–79. https://doi.org/10.1038/MT.2011.39.

Heo, Jeong, Tony Reid, Leyo Ruo, Caroline J Breitbach, Steven Rose, Mark Bloomston, Mong Cho, et al. 2013. “Randomized Dose-Finding Clinical Trial of Oncolytic Immunotherapeutic Vaccinia JX-594 in Liver Cancer.” *Nature Medicine* 19 (3): 329–36. https://doi.org/10.1038/nm.3089.

Hinrichs, Christian S, and Steven A Rosenberg. 2014. “Exploiting the Curative Potential of Adoptive T-Cell Therapy for Cancer.” *Immunological Reviews* 257 (1): 56–71. https://doi.org/10.1111/imr.12132.

Hirvinen, Mari, Maria Rajecki, Mika Kapanen, Suvi Parviainen, Noora Rouvinen-Lagerström, Iulia Diaconu, Petri Nokisalmi, Mikko Tenhunen, Akseli Hemminki, and Vincenzo Cerullo. 2015. “Immunological Effects of a Tumor Necrosis Factor Alpha–Armed Oncolytic Adenovirus.” *Human Gene Therapy* 26 (3): 134–44. https://doi.org/10.1089/hum.2014.069.

Hodi, F. Stephen, Steven J. O’Day, David F. McDermott, Robert W. Weber, Jeffrey A. Sosman, John B. Haanen, Rene Gonzalez, et al. 2010. “Improved Survival with Ipilimumab in Patients with Metastatic Melanoma.” *New England Journal of Medicine* 363 (8): 711–23. https://doi.org/10.1056/NEJMoa1003466.

Hofmann, Elisabeth, Stephanie Weibel, and Aladar A Szalay. 2014. “Combination Treatment with Oncolytic Vaccinia Virus and Cyclophosphamide Results in Synergistic Antitumor Effects in Human Lung Adenocarcinoma Bearing Mice.” *Journal of Translational Medicine* 12 (1): 197. https://doi.org/10.1186/1479-5876-12-197.

Jha, Babal K, Beihua Dong, Carvell T Nguyen, Irina Polyakova, and Robert H Silverman. 2013. “Suppression of Antiviral Innate Immunity by Sunitinib Enhances Oncolytic Virotherapy.” *Molecular Therapy* 21 (9): 1749–57. https://doi.org/10.1038/MT.2013.112.

Kaufman, Howard L., Frederick J. Kohlhapp, and Andrew Zloza. 2015. “Oncolytic Viruses: A New Class of Immunotherapy Drugs.” *Nature Reviews Drug Discovery* 14 (9): 642–62. https://doi.org/10.1038/nrd4663.

Kazandjian, Dickran, Daniel L Suzman, Gideon Blumenthal, Sirisha Mushti, Kun He, Meredith Libeg, Patricia Keegan, and Richard Pazdur. 2016. “FDA Approval Summary: Nivolumab for the Treatment of Metastatic Non-Small Cell Lung Cancer With Progression On or After Platinum-Based Chemotherapy.” *The Oncologist* 21 (5): 634–42. https://doi.org/10.1634/theoncologist.2015-0507.

Khodarev, N. N., B. Roizman, and R. R. Weichselbaum. 2012. “Molecular Pathways: Interferon/Stat1 Pathway: Role in the Tumor Resistance to Genotoxic Stress and Aggressive Growth.” *Clinical Cancer Research* 18 (11): 3015–21. https://doi.org/10.1158/1078-0432.CCR-11-3225.

Kirn, David H, Yaohe Wang, Fabrice Le Boeuf, John Bell, and Steve H Thorne. 2007. “Targeting of Interferon-Beta to Produce a Specific, Multi-Mechanistic Oncolytic Vaccinia Virus.” Edited by Grant McFadden. *PLoS Medicine* 4 (12): e353. https://doi.org/10.1371/journal.pmed.0040353.

Kuhn, Irene, Paul Harden, Maxine Bauzon, Cecile Chartier, Julie Nye, Steve Thorne, Tony Reid, et al. 2008. “Directed Evolution Generates a Novel Oncolytic Virus for the Treatment of Colon Cancer.” Edited by Dong-Yan Jin. *PLoS ONE* 3 (6): e2409. https://doi.org/10.1371/journal.pone.0002409.

Lasek, Witold, Radosław Zagożdżon, and Marek Jakobisiak. 2014. “Interleukin 12: Still a Promising Candidate for Tumor Immunotherapy?” *Cancer Immunology, Immunotherapy* 63 (5): 419–35. https://doi.org/10.1007/s00262-014-1523-1.

Lebel, Francois M., John A. Barrett, E. Antonio Chiocca, John Yu, Rimas Vincas Lukas, Seema Nagpal, Priya Kumthekar, Suma Krishnan, and Laurence JN Cooper. 2016. “Effect of Controlled Intratumoral Viral Delivery of Ad-RTS-HIL-12 + Oral Veledimex in Subjects with Recurrent or Progressive Glioma.” *Journal of Clinical Oncology* 34 (15_suppl): 2052–2052. https://doi.org/10.1200/JCO.2016.34.15_suppl.2052.

Leber, Mathias F, Sascha Bossow, Vincent HJ Leonard, Karim Zaoui, Christian Grossardt, Marie Frenzke, Tanner Miest, et al. 2011. “MicroRNA-Sensitive Oncolytic Measles Viruses for Cancer-Specific Vector Tropism.” *Molecular Therapy* 19 (6): 1097–1106. https://doi.org/10.1038/MT.2011.55.

Ledford, Heidi. 2015. “Cancer-Fighting Viruses Win Approval.” *Nature* 526 (7575): 622–23. https://doi.org/10.1038/526622a.

Lee, Daniel W, Rebecca Gardner, David L Porter, Chrystal U Louis, Nabil Ahmed, Michael Jensen, Stephan A Grupp, and Crystal L Mackall. 2014. “Current Concepts in the Diagnosis and Management of Cytokine Release Syndrome.” *Blood* 124 (2): 188–95. https://doi.org/10.1182/blood-2014-05-552729.

Li, Jun, Mark O’Malley, Julie Urban, Padma Sampath, Z Sheng Guo, Pawel Kalinski, Steve H Thorne, and David L Bartlett. 2011. “Chemokine Expression From Oncolytic Vaccinia Virus Enhances Vaccine Therapies of Cancer.” *Molecular Therapy* 19 (4): 650–57. https://doi.org/10.1038/MT.2010.312.

Lipson, E. J., and C. G. Drake. 2011. “Ipilimumab: An Anti-CTLA-4 Antibody for Metastatic Melanoma.” *Clinical Cancer Research* 17 (22): 6958–62. https://doi.org/10.1158/1078-0432.CCR-11-1595.

Lolkema, M. P., H.-T. Arkenau, K. Harrington, P. Roxburgh, R. Morrison, V. Roulstone, K. Twigger, et al. 2011. “A Phase I Study of the Combination of Intravenous Reovirus Type 3 Dearing and Gemcitabine in Patients with Advanced Cancer.” *Clinical Cancer Research* 17 (3): 581–88. https://doi.org/10.1158/1078-0432.CCR-10-2159.

Lopez, M. Verónica, Diego L. Viale, Eduardo G. A. Cafferata, Alicia I. Bravo, Cecilia Carbone, David Gould, Yuti Chernajovsky, and Osvaldo L. Podhajcer. 2009. “Tumor Associated Stromal Cells Play a Critical Role on

the Outcome of the Oncolytic Efficacy of Conditionally Replicative Adenoviruses." Edited by Alfred Lewin. *PLoS ONE* 4 (4): e5119. https://doi.org/10.1371/journal.pone.0005119.

Lundstrom, Kenneth. 2018. "New Frontiers in Oncolytic Viruses: Optimizing and Selecting for Virus Strains with Improved Efficacy." *Biologics: Targets and Therapy* 12: 43–60. https://doi.org/10.2147/BTT.S140114.

Mader, E. K., Y. Maeyama, Y. Lin, G. W. Butler, H. M. Russell, E. Galanis, S. J. Russell, A. B. Dietz, and K.-W. Peng. 2009. "Mesenchymal Stem Cell Carriers Protect Oncolytic Measles Viruses from Antibody Neutralization in an Orthotopic Ovarian Cancer Therapy Model." *Clinical Cancer Research* 15 (23): 7246–55. https://doi.org/10.1158/1078-0432.CCR-09-1292.

Maisonneuve, Charles, Sylvie Bertholet, Dana J Philpott, and Ennio De Gregorio. 2014. "Unleashing the Potential of NOD- and Toll-like Agonists as Vaccine Adjuvants." *Proceedings of the National Academy of Sciences of the United States of America* 111 (34): 12294–99. https://doi.org/10.1073/pnas.1400478111.

Marin-Acevedo, Julian A., Aixa E. Soyano, Bhagirathbhai Dholaria, Keith L. Knutson, and Yanyan Lou. 2018. "Cancer Immunotherapy beyond Immune Checkpoint Inhibitors." *Journal of Hematology & Oncology* 11 (1): 8. https://doi.org/10.1186/s13045-017-0552-6.

McCart, Andrea J., Navneet Mehta, Deborah Scollard, Raymond M. Reilly, Jorge A. Carrasquillo, Nan Tang, Hui Deng, et al. 2004. "Oncolytic Vaccinia Virus Expressing the Human Somatostatin Receptor SSTR2: Molecular Imaging after Systemic Delivery Using 111In-Pentetreotide." *Molecular Therapy* 10 (3): 553–61. https://doi.org/10.1016/J.YMTHE.2004.06.158.

Mell, Loren K., Kevin T. Brumund, Gregory A. Daniels, Sunil J. Advani, Kaveh Zakeri, Mary E. Wright, Sara-Jane Onyeama, et al. 2017. "Phase I Trial of Intravenous Oncolytic Vaccinia Virus (GL-ONC1) with Cisplatin and Radiotherapy in Patients with Locoregionally Advanced Head and Neck Carcinoma." *Clinical Cancer Research* 23 (19): 5696–5702. https://doi.org/10.1158/1078-0432.CCR-16-3232.

NCT03514836. 2018. "A Phase I/II, Safety Clinical Trial of DCVAC/PCa and ONCOS-102 in Men With Metastatic Castration-Resistant Prostate Cancer - Full Text View - ClinicalTrials.Gov." U. S. National Library of Medicine. 2018. https://clinicaltrials.gov/ct2/show/NCT03514836.

Nishio, N., I. Diaconu, H. Liu, V. Cerullo, I. Caruana, V. Hoyos, L. Bouchier-Hayes, B. Savoldo, and G. Dotti. 2014. "Armed Oncolytic Virus Enhances Immune Functions of Chimeric Antigen Receptor-Modified T Cells in Solid Tumors." *Cancer Research* 74 (18): 5195–5205. https://doi.org/10.1158/0008-5472.CAN-14-0697.

Patel, Daxa M., Paul M. Foreman, L. Burt Nabors, Kristen O. Riley, G. Yancey Gillespie, and James M. Markert. 2016. "Design of a Phase I Clinical Trial to Evaluate M032, a Genetically Engineered HSV-1 Expressing IL-12, in Patients with Recurrent/Progressive Glioblastoma Multiforme, Anaplastic Astrocytoma, or Gliosarcoma." *Human Gene Therapy Clinical Development* 27 (2): 69–78. https://doi.org/10.1089/humc.2016.031.

Pecora, Andrew L., Naiyer Rizvi, Gary I. Cohen, Neal J. Meropol, Daniel Sterman, John L. Marshall, Stuart Goldberg, et al. 2002. "Phase I Trial of Intravenous Administration of PV701, an Oncolytic Virus, in Patients with Advanced Solid Cancers." *Journal of Clinical Oncology* 20 (9): 2251–66. https://doi.org/10.1200/JCO.2002.08.042.

Poppers, J, M Mulvey, D Khoo, and I Mohr. 2000. "Inhibition of PKR Activation by the Proline-Rich RNA Binding Domain of the Herpes Simplex Virus Type 1 Us11 Protein." *Journal of Virology* 74 (23): 11215–21. https://doi.org/10.1128/JVI.74.23.11215-11221.2000.

Post, Dawn E., Eric M. Sandberg, Michele M. Kyle, Narra Sarojini Devi, Daniel J. Brat, Zhiheng Xu, Mourad Tighiouart, and Erwin G. Van Meir. 2007. "Targeted Cancer Gene Therapy Using a Hypoxia Inducible Factor–Dependent Oncolytic Adenovirus Armed with Interleukin-4." *Cancer Research* 67 (14): 6872–81. https://doi.org/10.1158/0008-5472.CAN-06-3244.

Prasad, Vinay, Victoria Kaestner, and Sham Mailankody. 2018. "Cancer Drugs Approved Based on Biomarkers and Not Tumor Type—FDA Approval of Pembrolizumab for Mismatch Repair-Deficient Solid Cancers." *JAMA Oncology* 4 (2): 157. https://doi.org/10.1001/jamaoncol.2017.4182.

Prickett, Todd D, Jessica S Crystal, Cyrille J Cohen, Anna Pasetto, Maria R Parkhurst, Jared J Gartner, Xin Yao, et al. 2016. "Durable Complete Response from Metastatic Melanoma after Transfer of Autologous T Cells Recognizing 10 Mutated Tumor Antigens." *Cancer Immunology Research* 4 (8): 669–78. https://doi.org/10.1158/2326-6066.CIR-15-0215.

Przepiorka, D., C.-W. Ko, A. Deisseroth, C. L. Yancey, R. Candau-Chacon, H.-J. Chiu, B. J. Gehrke, et al. 2015. "FDA Approval: Blinatumomab." *Clinical Cancer Research* 21 (18): 4035–39. https://doi.org/10.1158/1078-0432.CCR-15-0612.

Raedler, Lisa A. 2015. "Opdivo (Nivolumab): Second PD-1 Inhibitor Receives FDA Approval for Unresectable or Metastatic Melanoma." *American Health & Drug Benefits* 8 (Spec Feature): 180–83. http://www.ncbi.nlm.nih.gov/pubmed/26629287.

Rajani, Karishma, Christopher Parrish, Timothy Kottke, Jill Thompson, Shane Zaidi, Liz Ilett, Kevin G Shim, et al. 2016. "Combination Therapy With Reovirus and Anti-PD-1 Blockade Controls Tumor Growth Through Innate and Adaptive Immune Responses." *Molecular Therapy* 24 (1): 166–74. https://doi.org/10.1038/MT.2015.156.

Ramesh, N., Ying Ge, David L Ennist, Mingzhu Zhu, Mervat Mina, Shanthi Ganesh, P Seshidhar Reddy, and De-Chao Yu. 2006. "CG0070, a Conditionally Replicating Granulocyte Macrophage Colony-Stimulating Factor-Armed Oncolytic Adenovirus for the Treatment of Bladder Cancer." *Clinical Cancer Research* 12 (1): 305–13. https://doi.org/10.1158/1078-0432.CCR-05-1059.

Rehman, Hasan, Ann W. Silk, Michael P. Kane, and Howard L. Kaufman. 2016. "Into the Clinic: Talimogene Laherparepvec (T-VEC), a First-in-Class Intratumoral Oncolytic Viral Therapy." *Journal for ImmunoTherapy of Cancer* 4 (1): 1–8. https://doi.org/10.1186/s40425-016-0158-5.

Rommelfanger, D. M., P. Wongthida, R. M. Diaz, K. M. Kaluza, J. M. Thompson, T. J. Kottke, and R. G. Vile. 2012. "Systemic Combination Virotherapy for Melanoma with Tumor Antigen-Expressing Vesicular Stomatitis Virus and Adoptive T-Cell Transfer." *Cancer Research* 72 (18): 4753–64. https://doi.org/10.1158/0008-5472.CAN-12-0600.

Rudin, C. M., J. T. Poirier, N. N. Senzer, J. Stephenson, D. Loesch, K. D. Burroughs, P. S. Reddy, C. L. Hann, and P. L. Hallenbeck. 2011. "Phase I Clinical Study of Seneca Valley Virus (SVV-001),a Replication-Competent Picornavirus, in Advanced Solid Tumors with Neuroendocrine Features." *Clinical Cancer Research* 17 (4): 888–95. https://doi.org/10.1158/1078-0432.CCR-10-1706.

Russell, Stephen J., and Kah Whye Peng. 2017. "Oncolytic Virotherapy: A Contest between Apples and Oranges." *Molecular Therapy* 25 (5): 1107–16. https://doi.org/10.1016/j.ymthe.2017.03.026.

Sabado, Rachel Lubong, and Nina Bhardwaj. 2013. "Dendritic Cell Immunotherapy." *Annals of the New York Academy of Sciences* 1284 (1): 31–45. https://doi.org/10.1111/nyas.12125.

Sanjuán, Rafael, and Valery Z. Grdzelishvili. 2015. "Evolution of Oncolytic Viruses." *Current Opinion in Virology* 13: 1–5. https://doi.org/10.1016/j.coviro.2015.01.014.

Sanjuán, Rafael, Miguel R Nebot, Nicola Chirico, Louis M Mansky, and Robert Belshaw. 2010. "Viral Mutation Rates." *Journal of Virology* 84 (19): 9733–48. https://doi.org/10.1128/JVI.00694-10.

Sathaiah, M, P Thirunavukkarasu, M E O'Malley, M A Kavanagh, R Ravindranathan, F Austin, Z S Guo, and D L Bartlett. 2012. "Oncolytic Poxvirus Armed with Fas Ligand Leads to Induction of Cellular Fas Receptor and Selective Viral Replication in FasR-Negative Cancer." *Cancer Gene Therapy* 19 (3): 192–201. https://doi.org/10.1038/cgt.2011.77.

Schietinger, Andrea, Karin Schreiber, Mary Philip, Rebecca B. Liu, and Hans Schreiber. 2010. "Bystander Killing of Cancer Requires the Cooperation of CD4+ and CD8+ T Cells during the Effector Phase." *The Journal of Experimental Medicine* 207 (11): 2469–77. https://doi.org/10.1084/jem.20092450.

Scott, Andrew M, James P Allison, and Jedd D Wolchok. 2012. "Monoclonal Antibodies in Cancer Therapy." *Cancer Immunity* 12: 14. http://www.ncbi.nlm.nih.gov/pubmed/22896759.

Shafren, D R, D J Dorahy, R A Ingham, G F Burns, and R D Barry. 1997. "Coxsackievirus A21 Binds to Decay-Accelerating Factor but Requires Intercellular Adhesion Molecule 1 for Cell Entry." *Journal of Virology* 71 (6): 4736–43. http://www.ncbi.nlm.nih.gov/pubmed/9151867.

Shafren, Darren, Howard Kaufman, Andrew Zloza, Jennifer Bryan, Janice Mehnert, Mark Grose, Nashat Gabrail, et al. 2017. "Abstract CT026: Phase 1B Study of Intratumoral Coxsackievirus A21 (CVA21) and Systemic Pembrolizumab in Advanced Melanoma Patients: Interim Results of the CAPRA Clinical Trial." *Cancer Research* 77 (13 Supplement): CT026–CT026. https://doi.org/10.1158/1538-7445.am2017-ct026.

Sharma, Padmanee, and James P. Allison. 2015. "Immune Checkpoint Targeting in Cancer Therapy: Toward Combination Strategies with Curative Potential." *Cell* 161 (2): 205–14. https://doi.org/10.1016/J.CELL.2015.03.030.

Sharma, Padmanee, Klaus Wagner, Jedd D. Wolchok, and James P. Allison. 2011. "Novel Cancer Immunotherapy Agents with Survival Benefit: Recent Successes and next Steps." *Nature Reviews Cancer* 11 (11): 805–12. https://doi.org/10.1038/nrc3153.

Sova, Pavel, Xiao-Wei Ren, Shaoheng Ni, Kathrin M Bernt, Jie Mi, Nancy Kiviat, and André Lieber. 2004. "A Tumor-Targeted and Conditionally Replicating Oncolytic Adenovirus Vector Expressing TRAIL for Treatment of Liver Metastases." *Molecular Therapy* 9 (4): 496–509. https://doi.org/10.1016/J.YMTHE.2003.12.008.

Stephenson, K B, N G Barra, E Davies, A A Ashkar, and B D Lichty. 2012. "Expressing Human Interleukin-15 from Oncolytic Vesicular Stomatitis Virus Improves Survival in a Murine Metastatic Colon Adenocarcinoma Model through the Enhancement of Anti-Tumor Immunity." *Cancer Gene Therapy* 19 (4): 238–46. https://doi.org/10.1038/cgt.2011.81.

Tesfay, Mulu Z, Amber C Kirk, Elizabeth M Hadac, Guy E Griesmann, Mark J Federspiel, Glen N Barber, Stephen M Henry, Kah-Whye Peng, and Stephen J Russell. 2013. "PEGylation of Vesicular Stomatitis Virus Extends Virus Persistence in Blood Circulation of Passively Immunized Mice." *Journal of Virology* 87 (7): 3752–59. https://doi.org/10.1128/JVI.02832-12.

Thomas, Maria A, Jacqueline F Spencer, Karoly Toth, John E Sagartz, Nancy J Phillips, and William SM Wold. 2008. "Immunosuppression Enhances Oncolytic Adenovirus Replication and Antitumor Efficacy in the Syrian Hamster Model." *Molecular Therapy* 16 (10): 1665–73. https://doi.org/10.1038/MT.2008.162.

Toda, Masahiro, Samuel D. Rabkin, Hidefumi Kojima, and Robert L. Martuza. 1999. "Herpes Simplex Virus as an in Situ Cancer Vaccine for the Induction of Specific Anti-Tumor Immunity." *Human Gene Therapy* 10 (3): 385–93. https://doi.org/10.1089/10430349950018832.

Tomala, Jakub, and Marek Kovar. 2016. “IL-2/Anti-IL-2 MAb Immunocomplexes: A Renascence of IL-2 in Cancer Immunotherapy?” *OncoImmunology* 5 (3): e1102829. https://doi.org/10.1080/2162402X.2015.1102829.

Ueda, Yasunori, Michinori Ogura, Shigesaburo Miyakoshi, Takahiro Suzuki, Yuji Heike, Shuzo Tagashira, Satoru Tsuchiya, Kazuma Ohyashiki, and Yasushi Miyazaki. 2017. “Phase 1/2 Study of the WT1 Peptide Cancer Vaccine WT4869 in Patients with Myelodysplastic Syndrome.” *Cancer Science* 108 (12): 2445–53. https://doi.org/10.1111/cas.13409.

Villalona-Calero, Miguel A., Elaine Lam, Gregory A. Otterson, Weiqiang Zhao, Matthew Timmons, Deepa Subramaniam, Erinn M. Hade, et al. 2016. “Oncolytic Reovirus in Combination with Chemotherapy in Metastatic or Recurrent Non-Small Cell Lung Cancer Patients with KRAS-Activated Tumors.” *Cancer* 122 (6): 875–83. https://doi.org/10.1002/cncr.29856.

Walunas, Theresa L., Deborah J. Lenschow, Christina Y. Bakker, Peter S. Linsley, Gordon J. Freeman, Jonathan M. Green, Craig B. Thompson, and Jeffrey A. Bluestone. 1994. “CTLA-4 Can Function as a Negative Regulator of T Cell Activation.” *Immunity* 1 (5): 405–13. https://doi.org/10.1016/1074-7613(94)90071-X.

Waters, Alicia M., James M. Johnston, Alyssa T. Reddy, John Fiveash, Avi Madan-Swain, Kara Kachurak, Asim K. Bag, G. Yancey Gillespie, James M. Markert, and Gregory K. Friedman. 2017. “Rationale and Design of a Phase 1 Clinical Trial to Evaluate HSV G207 Alone or with a Single Radiation Dose in Children with Progressive or Recurrent Malignant Supratentorial Brain Tumors.” *Human Gene Therapy Clinical Development* 28 (1): 7–16. https://doi.org/10.1089/humc.2017.002.

Wilkinson, Michelle J, Henry G Smith, Gráinne McEntee, Joan Kyula-Currie, Tim D Pencavel, David C Mansfield, Aadil A Khan, Victoria Roulstone, Andrew J Hayes, and Kevin J Harrington. 2016. “Oncolytic Vaccinia Virus Combined with Radiotherapy Induces Apoptotic Cell Death in Sarcoma Cells by Down-Regulating the Inhibitors of Apoptosis.” *Oncotarget* 7 (49): 81208–22. https://doi.org/10.18632/oncotarget.12820.

Wong, R. J., Mei-Ki Chan, Zhenkun Yu, Teresa H Kim, Amit Bhargava, Brendon M Stiles, Brian C Horsburgh, et al. 2004. “Effective Intravenous Therapy of Murine Pulmonary Metastases with an Oncolytic Herpes Virus Expressing Interleukin 12.” *Clinical Cancer Research* 10 (1): 251–59. https://doi.org/10.1158/1078-0432.CCR-0197-3.

Wong, Siu-Fun. 2005. “Cetuximab: An Epidermal Growth Factor Receptor Monoclonal Antibody for the Treatment of Colorectal Cancer.” *Clinical Therapeutics* 27 (6): 684–94. https://doi.org/10.1016/J.CLINTHERA.2005.06.003.

You, Zongbing, Dagmar C Fischer, Xiaowen Tong, Annette Hasenburg, Estuardo Aguilar-Cordova, and Dirk G Kieback. 2001. “Coxsackievirus–Adenovirus Receptor Expression in Ovarian Cancer Cell Lines Is Associated with Increased Adenovirus Transduction Efficiency and Transgene Expression.” *Cancer Gene Therapy* 8 (3): 168–75. https://doi.org/10.1038/sj.cgt.7700284.

Yu, Shengnan, Anping Li, Qian Liu, Tengfei Li, Xun Yuan, Xinwei Han, and Kongming Wu. 2017. “Chimeric Antigen Receptor T Cells: A Novel Therapy for Solid Tumors.” *Journal of Hematology & Oncology* 10 (1): 78. https://doi.org/10.1186/s13045-017-0444-9.

Yuan, Zhong-Yu, Li Zhang, Su Li, Xiao-Zhou Qian, and Zhong-Zhen Guan. 2003. “[Safety of an E1B Deleted Adenovirus Administered Intratumorally to Patients with Cancer].” *Ai Zheng = Aizheng = Chinese Journal of Cancer* 22 (3): 310–13. http://www.ncbi.nlm.nih.gov/pubmed/12654194.

Zhang, Kecheng, Zheng Peng, Xiaohui Huang, Zhi Qiao, Xinxin Wang, Ning Wang, Hongqing Xi, et al. 2017.

"Phase II Trial of Adjuvant Immunotherapy with Autologous Tumor-Derived Gp96 Vaccination in Patients with Gastric Cancer." *Journal of Cancer* 8 (10): 1826–32. https://doi.org/10.7150/jca.18946.

Zhang, Zilai, Weiguo Zou, Jinhui Wang, Jinfa Gu, Yunkun Dang, Binghua Li, Lili Zhao, Cheng Qian, Qijun Qian, and Xinyuan Liu. 2005. "Suppression of Tumor Growth by Oncolytic Adenovirus-Mediated Delivery of an Antiangiogenic Gene, Soluble Flt-1." *Molecular Therapy* 11 (4): 553–62. https://doi.org/10.1016/J.YMTHE.2004.12.015.